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SHORT COMMUNICATION

PARTHENOGENESIS THROUGH FIVE GENERATIONS IN THE SCORPION *LIOCHELES AUSTRALASIAE* (FABRICIUS 1775) (SCORPIONES, ISCHNURIDAE)

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ABSTRACT. Females of *Liocheles australasiae* (Fabricius 1775) collected from a maleless population on Iriomote Island, Ryukyu, Japan, and separately reared in the laboratory have parthenogenetically produced five successive generations in seven years. Many individuals of the first generation collected in July 1994, gave birth to the second generations from 1994–1998, and some of the second generation gave birth to the third generation from 1997–1999. The fourth generations were born from 1999–2001, and the fifth generations were born in January–August 2001. Most females of all generations gave birth to about 20 neonates after approximately an eight-month pregnancy. In the ovary of a fourth generation female, as well as in those of most of the second generation females, there were growing embryos and a number of oocytes of various sizes, suggesting a possibility of the sixth generation or subsequent generations by parthenogenesis.

Keywords: Thelytokous parthenogenesis, successive generations, histological section

Among 1259 scorpion species described in the world (Fet et al. 2000), thelytokous parthenogenesis has been reported only in seven species (Matthiesen 1962, 1971; San Martín & Gambardella 1966; Makioka & Koike 1984, 1985; Zolessi 1985; Lourenço 1991; Lourenço & Cuellar 1994; Makioka 1993; Maury 1997; Lourenço et al. 2000). In some of these species, parthenogenesis has been presumed based on the absence of males in the populations (Lourenço 1991; Lourenço & Cuellar 1994), female biased sex ratios (Maury 1997) and all female neonates (Lourenço 1991; Lourenço & Cuellar 1999). However, one of the best evidences of parthenogenesis is an achievement of independent rearing, i.e., being raised alone without the presence of males, through successive generations. In only three scorpion species, has parthenogenesis been confirmed by means of independent rearing through three or four successive generations; this was seen in two buthids, *Tityus serrulatus* Lutz & Mello 1922 (Matthiesen 1962, 1971; San Martín & Gambardella 1966) and *T. bolivianus uruguayensis* (Borelli 1900) (Zolessi 1985) and an ischnurid, *Liocheles australasiae* (Fabricius 1775) (Makioka 1993).

Matthiesen (1962) found in *Tityus serrulatus* that three individuals of the second generation, born from the first field-collected generation, parthenogenetically gave birth to the third generations when reared separately. Later he reported that one of the third generation individuals, reared separately, gave birth to a total of 33 fourth generation offspring (Matthiesen 1971). In *T. serrulatus*, San Martín & Gambardella (1966) also reported that two second generation females gave birth to 22 third generation offspring, only two of which gave birth to eight fourth generation offspring. Zolessi (1985) stated that, for *T. bolivianus uruguayensis* he successively obtained second and third generations when females were reared separately, but he did not show the numbers of mothers and neonates. In *Liocheles australasiae* Makioka (1993) obtained many third generation offspring under separate rearing conditions. In these previous works, however, only a few of the second or third generations produced offspring particularly in the *Tityus* species, and no neonates of the third or the fourth generations became mature to produce offspring, leaving unanswered the ques-

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Figure 1.—Separate rearing in *Liocheles australasiae*. Scale = 2 cm.

tion as to whether parthenogenesis is an evolutionary strategy in these species or whether the phenomenon is limited to only a few generations. In the present study, we have attempted to answer this question in *L. australasiae* by rearing them through more than four generations.

A total of 413 females of *Liocheles australasiae* were collected from a maleless population (Makioka & Koike 1984, 1985; Makioka 1992a, 1992b, 1993) on Iriomote Island, one of the Ryukyu Islands, Japan, in July 1994. These specimens are deposited in the collections of the Biological Sciences of University of Tsukuba (TKB-anim. 1008–1421). First generations derived from the collected specimens were serially numbered and kept separate in glass vials (27 mm in diameter and 55 mm in height) with a piece of wet filter paper (Fig. 1) at 28 ± 1 °C in a dark incubator, and fed termites once a week.

The first instar juveniles of the second generation born from those females immediately climbed onto their mother's back, stayed there without eating for about a week (Fig. 2), and then molted into second instar juveniles (Fig. 3) which left their mother and took food by themselves. Each of the second instar juveniles was kept separate in a new glass vial soon after the first molt, and serially numbered. The juveniles were fed termites once a week, the number of which was varied with the scorpion's instar. We took special care not to give soldier termites to the young scorpions, because the soldiers can wound or kill young scorpions. All specimens were watered daily and their conditions (molt, parturition, death, etc.) were checked and recorded.

Ten females of the second generation that experienced parturitions at least once and a female of the fourth generation that experienced parturition once were dissected in physiological saline solution for histological observations. The ovaries were removed, fixed with Bouin's solution, dehydrated in a graded ethanol-*n*-butanol series, embedded in paraffin and serially sectioned at 5 μm thickness. The sections were stained with Mayer's hematoxylin and eosin, and the number of oocytes, embryos growing in the ovarian diverticula, and empty ovarian diverticula (remnants of previous parturitions) was counted under a light microscope.

A total of 147 of the 413 first-generation females experienced 165 parturitions to produce 1118 second generation offspring during the period from 1994–1998. From these second generation offspring, 46 females became mature and experienced 98 parturitions to produce 493 third generation offspring, 19 of which experienced 28 parturitions to produce 180 fourth generation offspring. From the fourth generations, only seven females became mature and gave birth to 78 fifth generation offspring through six parturitions.

The ovary of each female dissected consisted of three longitudinal and four transverse ovarian tubes, which bore many growing ovarian diverticula containing oocytes or embryos and empty ovarian diverticula which had lost their embryos in the previous parturitions. In adult ovaries, as described in previous papers (Makioka 1992a; Yamazaki & Makioka 2001), there were neither oogonia nor oocytes in the walls of the ovarian tubes and all the oocytes were contained in their own ovarian diverticula, waiting to develop into embryos.

In the ovary of the fourth generation female examined immediately after her death, there were 20 large empty ovarian diverticula that had newly lost

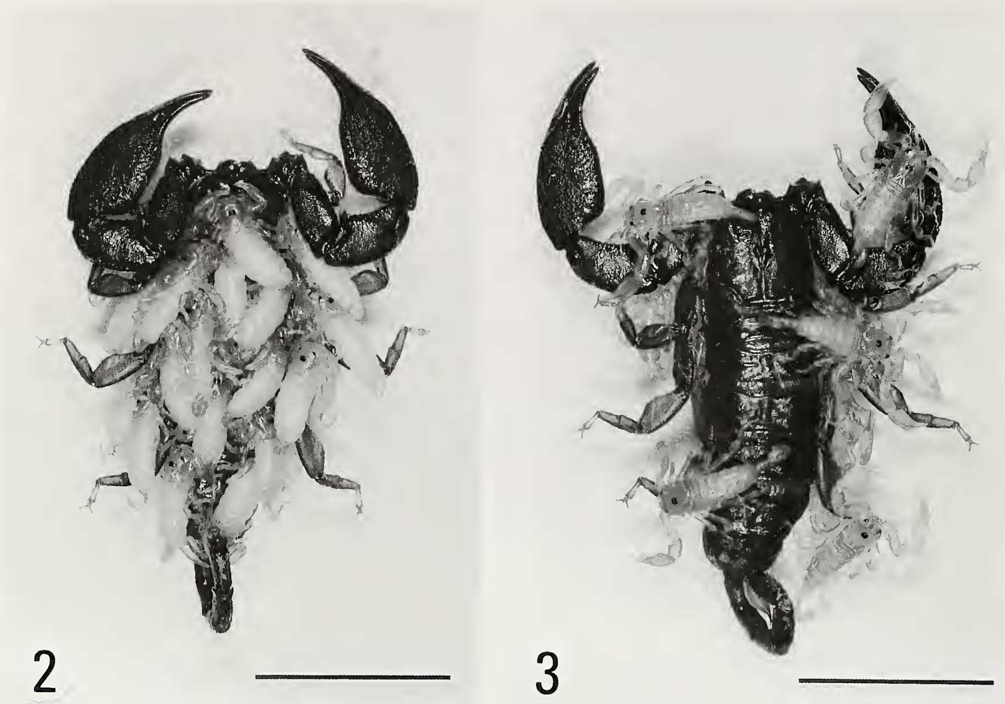
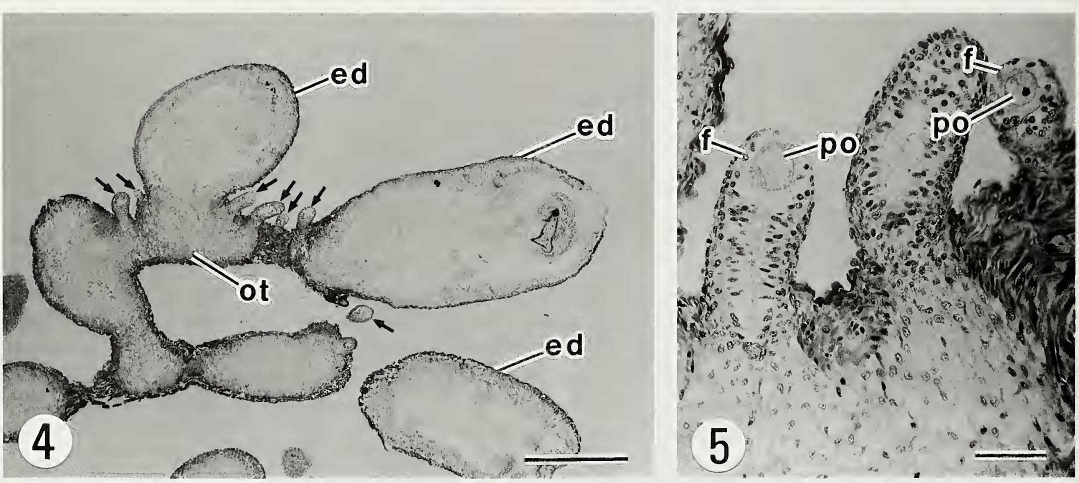


Figure 2–3.—First instar juveniles of *Liocheles australasiae* mounted on their mother's back. Scale = 1 cm. 3. Second instar juveniles of *Liocheles australasiae* immediately after the first molt on their mother's back. Scale = 1 cm.

their embryos at the last parturition (Fig. 4) and 86 smaller ovarian diverticula containing oocytes of various sizes (Fig. 5). No growing ovarian diverticula containing growing embryos were found. Two neonates corresponding to the difference in number between 20 empty ovarian diverticula and 18 neonates counted at birth, may have been eaten by the mother (see Polis & Sissom 1990). Neither male gonadal tissues, such as ovotestes, nor sperm receiving structures, such as spermathecae, were



Figures 4–5.—Ovarian sections of the fourth generation in *Liocheles australasiae*. 4. Ovarian diverticula containing a primary oocyte (arrows) and empty ovarian diverticula (ed). ot, ovarian tube. Scale: 500 μm. 5. Enlargement of small ovarian diverticula containing primary oocyte (po). f; follicle epithelium. Scale = 50 μm.

found in the ovaries of the second and the fourth generation females.

Some authors have attempted to rear scorpions from neonates to adults in order to ascertain their life histories (Rosin & Shulov 1963; Smith 1966; Matthiesen 1970; Francke 1976; Sissom & Francke 1983; Francke & Sissom 1984; Benton 1991; Brown 1997) and to confirm parthenogenetic reproduction (Matthiesen 1962, 1971; San Martín & Gambardella 1966; Zolessi 1985; Makioka 1993; Lourenço & Cuellar 1999). In most cases, however, none or only a few of neonates reached the adult stage (Matthiesen 1962, 1970, 1971; Rosin & Shulov 1963; San Martín & Gambardella 1966; Smith 1966; Francke 1976; Sissom & Francke 1983; France & Sissom 1984; Zolessi 1985; Benton 1991; Brown 1997). Among them, only three parthenogenetic scorpions successively continued to produce subsequent generations under the separate rearing conditions (Matthiesen 1962, 1971; San Martín & Gambardella 1966; Zolessi 1985; Makioka 1993).

Matthiesen (1971) succeeded in obtaining 33 neonates of the fourth generation from a mother of the third generation in *Tityus serrulatus* and Zolessi (1985) obtained an unspecified number of neonates of the third generation from the second generation in *T. bolivianus uruguayensis*, but they did not mention the fates of these neonates. In *Liocheles australasiae*, Makioka (1993) obtained a number of neonates of the third generation from four mothers of the second generation, but rearing was then terminated by an incubator accident. Thereafter, in 1994, we began the present study and have succeeded in obtaining a number of individuals of five successive generations of *L. australasiae*. At present, 78 second instars of the fifth generation are being raised. At the same time, in a fourth generation female after the first parturition, the ovary contained a number of oocytes of various sizes. It seems likely therefore, that the present specimens of *L. australasiae* will continue to reproduce parthenogenetically through further generations under the present laboratory conditions, as well as in their native population of Iriomote Island, and that our separate rearing method is well suited to *L. australasiae*. We suggest that because of the number of offspring produced by parthenogenesis and because of the number of generations we have now reared, parthenogenesis is indeed a stable reproductive strategy in this species.

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